# Tumour immunology: **T cells work together to fight cancer**

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**Recent studies have identified new melanoma antigens that are recognised by CD4+ T cells. Analysis of tumour-specific CD4+ T-cell responses may lead to the development of optimal anti-cancer vaccines that can induce an orchestrated effort of tumour-specific CD4+ and CD8+ T cells in the fight against cancer.**

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A central question in tumour immunology is whether a tumour-specific immune response is capable of controlling tumour progression in cancer patients. Although several animal models have established the role of tumour-specific CD4+ helper and CD8+ cytotoxic T cells in anti-tumour immunity, a definitive role for these cells in tumour-specific immunity in patients with cancer has yet to be shown. It is likely, however, that a successful anti-cancer vaccine will need to induce both of the components of protective immunity — antibody-mediated and cell-mediated immune responses.

To date, the identification of tumour-specific antigens has been focused almost exclusively on antigens recognised by tumour-specific CD8+ T cells, which are restricted to recognising antigens presented by major histocompatibility complex (MHC) class I molecules. Some of the antigens recognised by these T cells have been defined at the molecular level, by cloning tumour-specific CD8+ T cells and using them to screen gene expression libraries derived from tumour cell lines to find their targets [1]. Alternative strategies have relied on the elution of peptides from MHC class I molecules and the identification of antigenic peptides by a combination of chromatographic separation, analysis of recognition of chromatography fractions by tumour-specific CD8+ T cells and peptide sequencing [2]. Most of these tumour antigens were discovered in melanomas, because cell lines derived from this tumour type can be generated relatively easily from patient samples.

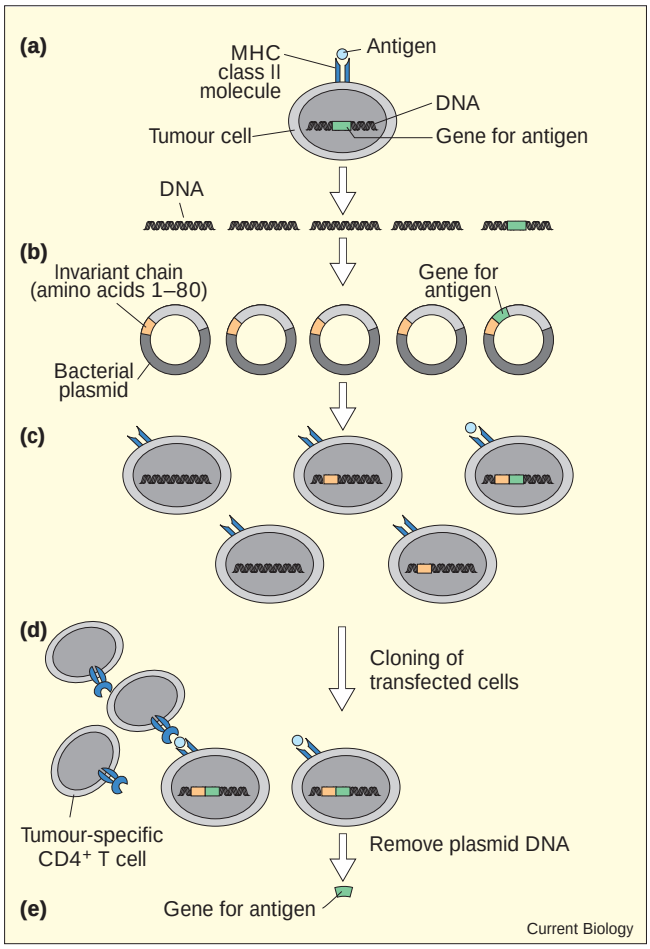
Recent data suggest that other tumours may also be susceptible to immune attack. In particular, the known repertoire of tumour antigens is rapidly expanding following the application of a new approach involving the screening of tumour-derived expression cDNA libraries for recognition by high-titre antibodies present in sera of the cancer patients. This new technique has identified a host of new antigens recognised by antibodies in cancer patients' sera. The fact that a proportion of the antigens isolated using this approach correspond to those identified in melanoma patients using CD8+-based techniques suggests that antibody responses against tumour antigens may be closely associated with CD8+ T-cell responses. Development of new methodologies capable of identifying as yet unknown tumour antigens is therefore extremely important for extending our understanding of the immune response to cancer. These techniques should also make it feasible to achieve the goal of defining a comprehensive repertoire of immunogenic gene products expressed by tumour cells.

Several recent papers have described new strategies to identify tumour antigens that are recognised by CD4+ T cells in the context of MHC class II molecules [4–8]. Animal models have established the central role played by CD4+ T cells in inducing tumour regression, even in the case of tumours that do not express MHC class II molecules. For example, vaccination of mice with recombinant vaccinia virus encoding the mouse melanocyte differentiation antigen Trp-1 led to the regression of the MHC class II negative mouse melanoma B16 [9]. This anti-tumour response was dependent on CD4+ T cells because tumour regression did not occur in mice deficient in MHC class II or in mice depleted of CD4+ T cells by injection of anti-CD4 monoclonal antibodies.

In another study, Ossendorp *et al.* [10] demonstrated that induction of tumour-specific CD4+ T cells resulted in protective immunity against virally induced MHC class II negative tumours. Tumour eradication, however, was dependent on the induction of tumour-specific CD8+ cytotoxic T cells, demonstrating the principle that CD8+ and CD4+ T-cell responses must act in concert to fight cancer. The activation of an antigen-specific CD8+ T-cell response by CD4+ T cells is dependent on the maturation of dendritic cells — highly specialised and efficient antigen-presenting cells. Interactions between CD40 expressed on the dendritic cell and its ligand, CD40L, expressed on CD4+ T cells [11] can lead to an amplification of the immune response and tumour destruction. Cytokines secreted by tumour-specific CD4+ T cells may also lead to tumour destruction by activating eosinophils and macrophages to produce superoxide and nitric oxide [12].

The mechanisms by which CD4+ T cells can be activated by MHC class II negative tumours and induce regression of MHC class II negative tumours are still ill defined. As shown for the induction of tumour-specific MHC class I

**Figure 1**




Outline of the strategy for isolating tumour antigens recognised by $CD4^+$ T cells. (a) Generation of a cDNA library from a tumour recognised by an MHC class II restricted $CD4^+$ T-cell clone. (b) Ligation of cDNA library downstream of the first 80 amino acids of the invariant chain. (c) Transfection of tumour cDNA library into target cells expressing appropriate MHC class II molecules. (d) Identification of target cells capable of activating the tumour-specific $CD4^+$ T-cell clone. (e) Identification of tumour antigen.

restricted responses [13], induction of MHC class II restricted responses specific for MHC class II negative tumours is controlled by the processing and presentation of tumour proteins by infiltrating dendritic cells. The findings of Ossendorp *et al.* [10] demonstrated that this mechanism of presentation, defined as cross-presentation, is also required for the recruitment of anti-tumour effector cells and the activation of tumour-specific $CD8^+$ T cells during the rejection of MHC class II negative tumours.

Constitutive expression of MHC class II molecules is often observed in human melanomas, which raises the possibility of whether MHC class II positive tumours may be directly involved in presenting intracellular antigenic proteins to $CD4^+$ T cells. Can intracellular tumour proteins have access to the MHC class II presentation pathway and be presented by the endogenous MHC class II molecules in the tumour? Analysis of the peptides eluted from MHC class I and class II molecules showed that there are two distinct intracellular pools from which MHC class I and class II molecules can obtain their peptide ligands. The majority of peptides bound to MHC class I molecules are derived from cytosolic proteins, whereas the majority of peptides loaded onto MHC class II molecules are derived from glycoproteins or secreted proteins.

This dichotomy of antigen presentation by MHC class I and class II molecules is central to the current understanding of the T-cell-mediated immune response. It has been shown, however, that intracellular proteins can actually be directed into the endosomal compartments for presentation by MHC class II molecules [14]. Lysosomes are capable of degrading cytosolic proteins by several mechanisms, including non-specific phagocytosis of cytosolic proteins, defined as autophagy, and specific selective protein import pathways for cytosolic proteins [15]. As these pathways are especially active in cells deprived of serum or in tissues of starved animals, it is possible that the processing of tumour proteins and presentation of the resulting peptides by endogenous MHC class II molecules in tumours may be more efficient in conditions of limited blood supply.

Wang and colleagues [4,5] have described a new strategy for the identification of tumour antigens presented by MHC class II molecules. This approach extends a strategy originally developed by van der Bruggen *et al.* [1] to identify MHC class I restricted tumour epitopes because it directs tumour antigens into the MHC class II presentation pathway. This pathway of presentation involves the binding of MHC class II molecules to a molecule termed the invariant chain in the lumen of the endoplasmic reticulum. These complexes are subsequently targeted into the endosomal compartment via a motif expressed in the first 80 amino acids of the invariant chain. Wang and colleagues generated a cDNA library from tumour cells that were capable of stimulating proliferation of an MHC class II restricted $CD4^+$ T-cell line (Figure 1). MHC class II positive cells were then transfected with this cDNA library. To ensure presentation by MHC class II molecules, the tumour-derived cDNA library had been cloned downstream of a gene fragment encoding the first 80 amino acids of the invariant chain. A similar approach was also used by Chaux *et al.* [6] to identify a $CD4^+$ T-cell response specific for the tumour antigen MAGE-3 by expressing full-length MAGE-3 in a retroviral construct encoding the first 80 amino acids of the invariant chain.

Application of this strategy has so far led to the identification of three MHC class II restricted epitopes: an epitope contained within a mutated form of the human CDC27

protein [4]; an epitope from a mutated form of triosephosphate isomerase [8]; and an epitope from a protein generated by the gene fusion of a low density lipid receptor gene and fucosegalactoside-fucosyltransferase [5]. It is of interest that the mutated form of CDC27 carries a mutation that affects the phosphorylation site Ser711. This site is encoded 50 amino acids upstream of the MHC class II epitope, which corresponds to residues 760–771 and is conserved between the wild-type and mutated CDC27 protein [4]. As a result of the Ser711 mutation, a large proportion of the CDC27 protein is localised in the cytosol, whereas wild-type CDC27 is mainly localised in the nucleus. Cytosolic localisation of the mutated CDC27 protein may account for the presentation of the MHC class II epitope, because tumour-specific $CD4^+$ T cells recognised cells transfected with the mutated CDC27 but not cells transfected with wild-type CDC27. These results illustrate the principle that mutations or post-translational modifications of cytosolic proteins may alter their processing and presentation and lead to the generation of MHC class I and class II epitopes. These findings may have important implications for the generation of tumour-specific antigens recognised by $CD8^+$ and $CD4^+$ T cells.

The application of the strategy described in these recent papers will undoubtedly speed up the identification of new tumour-specific antigens and facilitate the generation of cancer vaccines capable of stimulating both $CD4^+$ and $CD8^+$ T-cell responses. To optimise the immunogenicity of cancer vaccines it will also be important to improve the monitoring of the immune response. The use of tetrameric soluble peptide–MHC class I complexes ('tetramers') to identify tumour-specific $CD8^+$ T cells has now shown that these reagents allow rapid and accurate analysis of human $CD8^+$ T-cell responses in cancer patients [16]. The development of MHC class II tetramers will prove to be an invaluable tool for the monitoring of tumour-specific $CD4^+$ T-cell responses and the development of maximally immunogenic cancer vaccines.

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